ACS Photonics

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Taking Two-Photon Excitation to Exceptional Path-Lengths in Photonic Crystal Fiber

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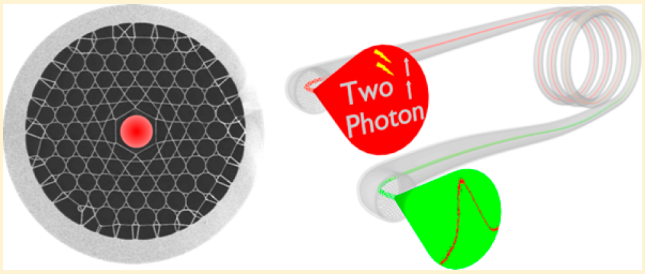
Supporting Information

ABSTRACT: The well-known, defining feature of two-photon excitation (TPE) is the tight, three-dimensional confinement of excitation at the intense focus of a laser beam. The extremely small excitation volume, on the order of 1 μm^3 (1 femtoliter), is the basis of far-reaching applications of TPE in fluorescence imaging, photodynamic therapy, nanofabrication, and three-dimensional optical memory. Paradoxically, the difficulty of detecting photochemical events in such a small volume is a barrier to the development of the two-photon-activated molecular systems that are essential to the realization of such applications. We show, using two-photon-excited fluorescence to directly visualize the excitation path, that confinement of both laser beam and sample solution within the 20 μm hollow core of a photonic crystal fiber permits TPE to be sustained over an extraordinary path-length of more than 10 cm, presenting a new experimental paradigm for ultrasensitive studies of two-photon-induced processes in solution.



KEYWORDS: *two-photon absorption, two-photon cross-section, nonlinear optics, fluorescence, photochemistry, optofluidics*

Two-photon excitation (TPE), once the preserve of esoteric molecular spectroscopy, is now widely used in biomedical fluorescence imaging[1−4] and is being highlighted for applications in photodynamic therapy,[5−7] three-dimensional (3D) optical data storage,[8−10] and nanofabrication.[11] The extremely high, local photon intensity required to achieve two-photon absorption (TPA) is created by focusing a pulsed laser beam into a spot of about 1 μm in diameter, giving a focal volume on the order of 1 femtoliter. The 3D confinement of excitation to the laser focal point (Figure 1a) is inherent to the TPE modality and is the basis of its applications.

Emerging applications of TPE are driving the synthesis and characterization of new chromophores with high TPA cross-sections and optimized photophysical or photochemical properties.[3,4,8−15] Paradoxically, the miniscule excitation volume makes the study of two-photon-induced photochemistry very challenging. The minute quantity of photoproducts generated in the femtoliter reaction volume cannot be analyzed *in situ* by conventional spectroscopic or analytical techniques. The accumulation of sufficient photoproduct for analysis requires lengthy irradiation of a stirred solution,[14−17] precluding detection of short-lived photoproducts or intermediates, fast kinetic measurements, and the study of thermally reversible reactions. Comprehensive analysis of the photochemical mechanism and products is crucial to the development of safe and effective photoactive drugs and cannot be assumed to be the same for one-photon and two-photon processes. The difference in selection rules and the tendency of the high instantaneous photon flux to induce excited-state absorption can result in different photochemical pathways for TPE.

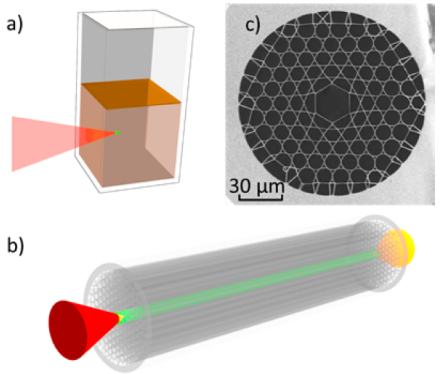


Figure 1. (a) In a bulk solution two-photon excitation of fluorescence is confined to the tight focus of a laser beam. (b) In a solution contained in the hollow core of a photonic crystal fiber, two-photon excitation is supported over a path-length of >10 cm, and fluorescence is collected along the entire path length. (c) Scanning electron micrograph of the cross-sectional structure of the hollow-core photonic crystal fiber.

Received: June 23, 2014
Published: August 25, 2014

   dx.doi.org/10.1021/ph5002236 | *ACS Photonics* 2014, 1, 790−793

We now take advantage of a radical development in optical fiber technology, the hollow-core photonic crystal fiber (HC-PCF),[18] to achieve TPE of solution-phase samples over a path-length of >10 cm, 5 orders of magnitude greater than that in the conventional excitation regime, opening the door to ultrasensitive, *in situ* spectroscopic monitoring of two-photon photochemistry.

In HC-PCF, light is trapped in the hollow core by the surrounding 2D periodic "photonic crystal" cladding, as illustrated in Figure 1b and c. This permits the infiltration of a sample of gas or liquid into the hollow core while maintaining the high optical transmission efficiency of the fiber (Figure 1b). Exploitation of the intense, long-path-length light−matter interactions within HC-PCF has concentrated primarily on the achievement of ultrafast nonlinear processes in gas-filled fibers, in the realm of quantum optics.[19,20] However, recent studies on intrafiber excitation of solution-phase samples have started to reveal the potential of HC-PCF as an optofluidic system for chemical sensing and photochemical applications.[21] To realize intrafiber TPE of fluorescence, we fabricated a custom-designed HC-PCF that guides efficiently over a wide spectral range to encompass both the excitation beam and the emitted fluorescence.

The PCF-based optofluidic system is illustrated in Figure 2. The 810 nm beam from the mode-locked Ti:sapphire laser is

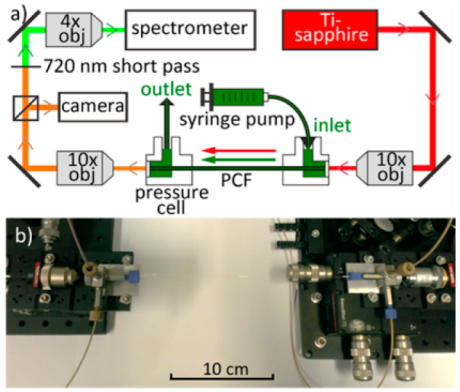


Figure 2. Schematic layout (a) and photograph (b) of the experimental system.

coupled, by a microscope objective, into the hollow core of the PCF, which contains the fluorophore solution. Fluorescence is collected at the output of the fiber by a second objective, filtered using a 720 nm short-pass filter to remove the excitation beam, and conducted to a spectrometer. A CCD camera monitors the excitation beam at the output of the fiber to aid alignment. The 30 cm length of the sample fiber is mounted between two custom-built pressure cells to permit introduction of the sample solution into the fiber, by a syringe pump, without loss of optical alignment. (Further experimental details are given in the Supporting Information.)

The focused Ti-sapphire laser beam is tightly confined in the 22-μm-diameter, hollow core of the fiber (Figure 3a and c), maintaining a high excitation intensity along the length of the fiber. The drop in laser peak intensity along the length of the fiber can be predicted by taking account of intrinsic fiber losses, solvent absorption, and pulse broadening (see Supporting Information). The theoretical decrease in laser intensity with increasing path-length along the fiber is plotted in Figure 4, together with the predicted decrease in the TP-excited emission

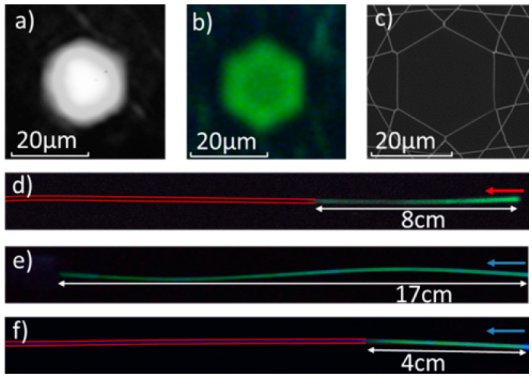


Figure 3. (a) Guided Ti-sapphire laser mode imaged at the HC-PCF output. (b) Two-photon-excited fluorescence imaged at the HC-PCF output. (c) Scanning electron micrograph of the HC-PCF end face. (d) Side-view of the HC-PCF, showing two-photon-excited fluorescence of fluorescein along a length of 8 cm. (e) Side-view of one-photon-excited fluorescein fluorescence along a 17 cm length of HC-PCF. (f) Side view of one-photon-excited fluorescence in an HC-PCF in which only a short section, at the in-coupling end, contains fluorescein. In images d−f, the excitation beam is coupled into the right-hand end of the fiber. In images d and f, the portion of the fiber from which there is no detectable fluorescence leakage is outlined in red for the purpose of illustration.

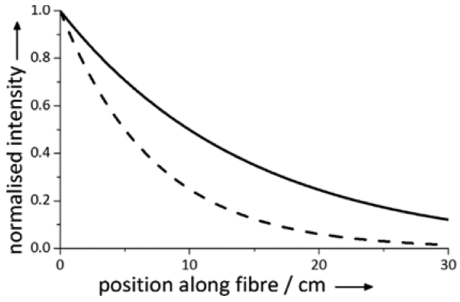


Figure 4. Predicted dependence of excitation laser intensity (top, solid curve) and two-photon-excited emission intensity (bottom, dashed curve) on position, along the length of the PCF.

intensity as a function of position along the fiber, assuming a quadratic relation between emission intensity and excitation intensity. It is evident that TPE should be easily sustainable over a path-length of several centimeters, at least.

The path-length over which TPE can be sustained was determined by photographing the emission escaping from the perimeter of an HC-PCF filled with an aqueous solution of fluorescein (Figure 3d). The fluorescence that is emitted within the hollow core is efficiently collected and guided by the fiber to emerge at the end face (Figure 3b), but, at the point of excitation, where the emission is isotropic, photons emitted in directions outside the acceptance angle of the fiber can escape through the cladding. The intensity of emission being excited at a particular point can be gauged, therefore, by the leakage intensity at that point. In Figure 3d, escape of fluorescence from the fiber is seen clearly over a distance of about 8 cm (plus an additional 3.5 cm, which is concealed in the sample-introduction cell) from the point of in-coupling of the laser. Therefore, TPE has been maintained over more than 10 cm. The drop in fluorescence intensity along the fiber is quantitatively consistent with that predicted in Figure 4, as shown in Supporting Information Figure S6. For comparison, Figure 3e shows a fluorescein-filled fiber subjected to one-

dx.doi.org/10.1021/ph5002236 | ACS Photonics 2014, 1, 790−793

photon excitation (OPE) at 470 nm. Here, a gradual decrease in intensity is seen along the fiber length, due to the cumulative absorption of the excitation light by fluorescein, in accord with the Beer–Lambert law. Finally, Figure 3f shows OPE of a fiber in which only a short section, at the in-coupling end, has been infiltrated with fluorescein; the remaining length of the core contains water. Leakage of fluorescence is seen only along the section that contains the fluorophore, although fluorescence is guided along the full length of the fiber to the output end. This confirms that the observed leakage arises from fluorescence at the point of excitation, not from escape of guided fluorescence that originated from excitation closer to the laser input.

Conventional measurements of two-photon-excited fluorescence in solution typically use laser peak irradiance in the range 10^8 to 10^{11} W cm^{-2} and fluorophore concentrations around 10^{-5} M.[22−25] As illustrated in Figure 5a and b, TPE within the

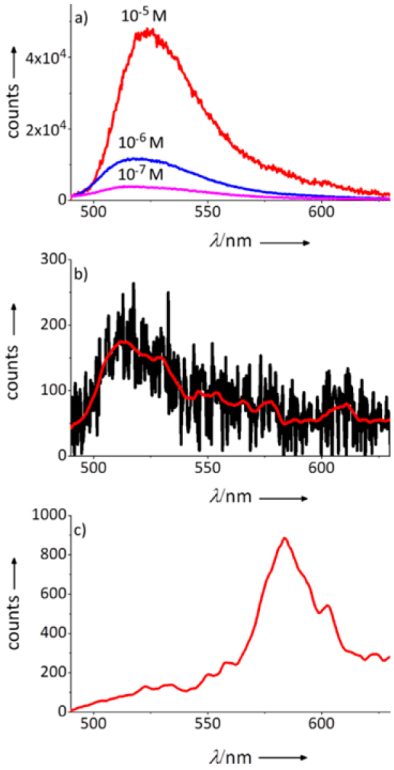


Figure 5. Two-photon-excited fluorescence spectra of fluorescein (a) for concentrations from 10^{-5} to 10^{-7} M and (b) for a 10^{-9} M solution. Raw data (500 ms integration time) are shown in black, and smoothed spectrum is shown in red. (c) Two-photon-excited fluorescence spectrum (smoothed) of 10^{-5} M rhodamine B, using a 150 mW CW laser as excitation source.

PCF enables measurements at concentrations as low as 10^{-9} M, at an irradiance of 9×10^8 W cm^{-2} (average power of 30 mW). Measurement of a quadratic dependence of emission intensity on excitation power (Figure 6) confirmed that the observed fluorescence was, indeed, the result of TPA. The increase in detection sensitivity of greater than 4 orders of magnitude for intrafiber excitation correlates well with the increase in TPE path length from ∼1−10 μm in conventional experiments to >10 cm in the PCF. The detection sensitivity is limited by the intrinsic noise of the low-cost CCD detector used here and could be improved further by photon-counting detection.

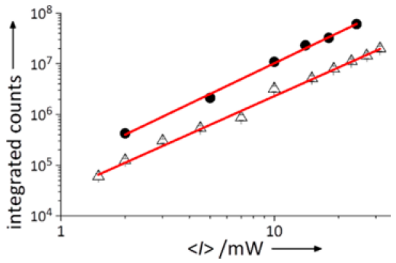


Figure 6. Log–log plot demonstrating a quadratic dependence of emission intensity on incident average laser power at 810 nm, for 10^{-5} M solutions of fluorescein (△) and rhodamine B (●). The fitted lines have a gradient of 1.89 and 2.01, respectively.

The results of similar TPE experiments on a second fluorophore, rhodamine B (RhB), are shown in Figures 5c and 6. Using the data in Figure 6, the fluorescence TPE cross-section (the TPA cross-section multiplied the fluorescence quantum yield) of RhB was determined to be 4.0 times that of fluorescein, at 810 nm. This leads to a value of 9.1 for the ratio of the TPA cross-sections of RhB and fluorescein, in good agreement with the reported absolute TPA cross-section values at 810 nm of 260 and 32 GM for RhB and fluorescein, respectively.[26] Thus, the PCF modality offers a straightforward method for the measurement of relative TPE cross-sections on subpicomole sample quantities, making it highly attractive for the assessment of newly developed fluorophores, which, as products of research-scale synthesis, are usually available only in small quantities.

Although the mode-locked Ti-sapphire laser is the standard TPE source, a few reports show the use of continuous-wave (CW) lasers to achieve TPE, by focusing several hundreds of millliwatts into a submicrometer spot.[27,28] In the PCF, we were able to record the two-photon-excited fluorescence spectrum of RhB using the Ti:sapphire laser in CW mode at a modest power of 150 mW (Figure 5c). Near-infrared diode lasers with CW output powers in this range are now readily available, offering an alternative low-cost, compact excitation source for intrafiber TPE.

The ability of HC-PCF to support TPE over a path-length of >10 cm overturns the common perception that TPE occurs only over submillimeter distances at the focal point of a laser beam, with important implications for the study of TP-induced photochemistry. We have already demonstrated the efficacy of PCF for ultrasensitive *in situ* study of one-photon photochemistry by intrafiber, long-path-length absorption spectroscopy,[29,30] and it is now evident that the same approach can be applied to two-photon photochemistry. Moreover, the containment of photochemical products within the core of the PCF facilitates their transfer into analytical instruments for in-line analysis and identification. For example, we have directly injected photochemical products from a PCF into an electrospray source for mass spectrometry.[31] In conclusion, the unique optofluidic environment within the HC-PCF presents a new experimental paradigm for the study of two-photon photophysics and photochemistry that will hasten the translation of TPE from the research laboratory into practical applications in clinical medicine, optical computing, and nanotechnology.

dx.doi.org/10.1021/ph5002236 | *ACS Photonics* 2014, 1, 790−793

■ ASSOCIATED CONTENT

Supporting Information

Details of the experimental procedures; theoretical treatment of fiber losses and pulse dispersion. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to the Koerber Foundation (Germany) and the EPSRC (UK) for financial support. G.O.S.W. is a recipient of an EPSRC Prize Postdoctoral Fellowship.

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